

THE ROLE OF MUCINOLYTIC BACTERIA IN FEEDLOT BLOAT

by

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INTRODUCTION

Bloat, in almost all instances, is a complex nutritional and physiological disorder of ruminants resulting from the production of gas of uncontrollable nature by the rumen microbial fermentation. This gas is trapped within rumen ingesta forming a froth or foam and, thus, cannot be released by the normal eructation mechanism. Bloat was observed as early as 60 A. D. by the Romans. The incidence of this disease has probably increased during recent years due to intensive feeding and pasturing practices, to the large increase in legume pasture acreage, and to a more intensive pasture fertilization program.

Bloat is an economic problem in countries where an intensive pasture program is practiced. The losses from bloat include death of 10 to 20 per cent of the bloated animals, loss of milk yield, and loss of meat production. Possibly the most serious factor is the loss of excellent legume pastures since after the death of one or two animals the entire herd grazing on the pasture is removed. Also, due to fear of bloat less productive grasses are used in place of legumes. Other losses include loss of manpower to attend the affected cattle, expensive treatment, and the heavy expenditure on research for solution of the problem. The losses of production of pastures and loss of cattle are estimated to be about 40 million dollars annually in the United States alone (Parham and Griffith, 1956).

The earlier concept of the nature of bloat which suggested that the distention of the rumen and reticulum was due to an accumulation of free gas has been clarified during recent years. It has been observed that the condition is complex in nature and gas resulting from rumen fermentation is trapped within the rumen contents to form a stable foam with the presence of very

little free gas at the dorsum of the rumen.

Stable froth forms in both legume and feedlot types of bloat. The factors involved in the formation of froth may be divided into three categories:

1. Those concerned with the physical nature and chemical constituents of bloat provoking plants, particularly proteins, hemi-celluloses, reducing sugars, saponins, pectins, and galacturonic acids obtained on hydrolysis of pectic substances.
2. Animal factors such as heredity, rate of flow and composition of saliva, and failure of the eructation mechanism due to toxins or derangement of the nerves controlling it.
3. Microorganisms associated with froth formation by producing slimes or by destroying natural antifoaming agents which may be present in the rumen.

The role of saliva as a bloat preventive was reported by Weiss, 1953. This has been established recently by results of work conducted at the Kansas station. The mucin of saliva was shown to act as an antifoaming agent in vitro. Animal mucins introduced into the rumen of cattle grazing alfalfa prevented bloat for four hours.

Since the bloat preventive ability of mucin was observed to be effective only for a few hours, it was assumed that the antifoaming action of mucin might be lost due to mucin degradation by the enzymes produced by certain species of rumen microorganisms. Consequently an attempt was made at this station to determine whether mucinolytic organisms existed in the rumen.

Hay (1961) isolated several organisms which could degrade salivary mucin. In preliminary studies, when cultures of these organisms were introduced into the rumens of fistulated cows grazing a mature nonbloat provoking alfalfa pasture, bloat resulted in the majority of instances. In the light of these

results, studies were initiated to determine whether similar mucinolytic organisms would be able to provoke bloat in cattle fed feedlot rations.

REVIEW OF LITERATURE

Bloat is a confusing entity in which plants, animals, and microorganisms are implicated. Several theories have been put forward as to its cause although none of these has proved satisfactory. It has been believed that more than one factor is involved in the process of foaming of the ruminal contents. When the volume and stability of the foam is markedly increased, bloat may result. All animals are not equally susceptible to bloat and all the plants do not have equal bloat provoking ability. Animals bloating on a particular pasture today may not bloat tomorrow and vice versa. Bloat in general and acute bloat in particular comes with little warning or predictability.

Considerable effort has been directed towards the elucidation of the processes of formation of stable froth in the bloating cow. In spite of a vast amount of information available in this field, as yet none of the theories presented regarding the etiology of the condition has been completely substantiated.

Udall (1947) described the symptoms of bloat as being the distention of the rumen and the reticulum. Hutyra, Marek, and Manninger (1946) defined acute bloat in ruminants as a "rapid distention of the rumen and reticulum due to the formation of gas." Wooldridge (1934) defined bloat as a distention of the rumen by gas formation as a result of fermentation of the contents of the organ. All these workers agree with the concept that bloat is the result of an accumulation of gas in the rumen and reticulum.

Bloat has been classified in several ways: (a) pasture and feedlot,

(b) primary and secondary, and (c) acute, subacute, and chronic.

Pasture bloat results when ruminants graze on very succulent pasture, particularly young, rapidly growing legumes in the pre-bloom stage. The disease occasionally occurs when cattle are grazed on cereal crops, rape, cabbages, peas, beans, and grass pastures.

The common legumes have been classified according to their bloat provoking potential in the following order: alfalfa 108, Ladino clover 100, red clover 83, white dutch clover 64, and crimson clover 7 (Blood and Henderson, 1960).

Feedlot bloat, which is also of the frothy type with varying amounts of free gas in the rumen, is observed mostly in beef cattle and fattening sheep fed on high concentrate rations with or without restricted amounts of roughage (Blood and Henderson, 1960). This condition is also prone to occur when finely ground feeds are fed. The severity of feedlot bloat progresses with time and, thus, may differ from legume bloat in this respect. Feedlot bloat may be subacute or acute.

Primary bloat in ruminants signifies pasture bloat or legume bloat which may vary in intensity. Invariably, cattle grazing on lush leguminous pasture develop bloat which does not manifest distressing symptoms like difficulty in respiration, frequent urination, defecation, etc. This condition is referred to as subacute bloat and the ruminal pressure, as measured by the tympanometer, varies from slightly above zero to 57 mm.Hg. (Cole and Boda, 1960). The aggravation of subacute bloat associated with the distressing syndrome is called acute bloat and the ruminal pressure varies from 45 to 69 mm.Hg.

Secondary bloat or chronic bloat is not a disease per se, but is observed in the course of various afflictions of the gastrointestinal tract. This occurs in ruminal stony, traumatic gastritis or reticulitis, obstruction of

gastric orifices by hair or feed balls, ulcers of the abomasum, stenosis of the esophagus, stomach worms, peritonitis, and sometimes due to pressure on the vagus nerve. Chronic bloat may occur irrespective of feed.

Rumen fermentation is the natural process inherent to ruminants which is carried out by many different types of microorganisms, both fauna and flora. These organisms act on feed with the consequent production of different fatty acids and a large amount of gases. The composition of rumen gases varies according to the dietary regimen, rumen microbial status, time after feeding, etc. The analyses of gases produced by cows on alfalfa pasture are as follows: 67 per cent CO_2 ; 26 per cent CH_4 ; 7 per cent $\text{N}_2 + \text{H}_2$; 0.1 per cent H_2S (Kleiber, Cole, and Mead, 1943).

The pathogenesis of legume bloat is of prime importance. The main two groups of factors involved are formation of foam in the rumen and reticulum, and atony of the rumen. Pasture bloat or feedlot bloat seems not to be accompanied by ruminal atony (Cole and Mead, 1943). In fact, in the early stages of bloat, hypermotility of the rumen is observed. In almost all cases of bloat the gas is mixed intimately with the solid and liquid ruminal contents and forms dense froth. Under normal conditions the gases produced in the reticulo-rumen are expelled by the process of eructation. The sequence of events is as follows: The process begins with two contractions of the reticulum which results in emptying of the organ followed by relaxation of the relatively empty reticulum. Contraction of the rumino-reticular fold up to the level of the cardia prevents the ingesta from going back to the reticulum. The two caudal sphincters close and the cranial esophageal sphincter opens. Contraction of the rumen pushes the gases forward around the cardia down to the reticulum and finally the gases are pushed through the relaxed cranial esophageal sphincter into the atmosphere due to relaxation of cardiac and

diaphragmatic sphincters and increased intrathoracic pressure (Dougherty, 1955).

Factors Involved in the Production of Foam

Proteins. Cytoplasmic proteins of legumes and mucoproteins of saliva are thought to be foaming agents. Johns, Mangan, and Reid (1957) stated that the cytoplasmic proteins of plants are the chief offenders in the production of foam. Mangan (1958) observed that the cytoplasmic protein of red clover had optimum foam strength at pH 5.4 to 6.0 which is nearly the normal pH of the rumen content. He observed that the cytoplasmic protein of red clover was released rapidly in large amounts and the concentration raised to 20 mg. N per 100 ml. of rumen liquor one hour after the commencement of feeding red clover. This soluble protein at appropriate salt concentrations formed very strong and stable foam. In contrast to these observations, Ferguson and Terry (1955) suggested that the plant proteins are not so important in the production of stable foam. Boda et al. (1957) reported that the bloat provoking ability of alfalfa hay is lost or minimized due to denaturation of cytoplasmic proteins and they agree with the view that proteins of green legumes are of importance in the etiology of bloat. Bartley and Bassette (1961) detected 15 amino acids in frothy ruminal content and suggested that proteins are involved in the composition of stable foam in bated animals.

Boda et al. (1957) fed dehydrated alfalfa hay to cows and drenched them with four liters of fresh egg white. Moderate bloat developed in the cows. In contrast to the above view, Allen et al. (1960) reported that addition of egg white to alfalfa hay did not influence bloat. Cole and Mead (1943) also reported that a number of grasses in their early stages of growth contain a large quantity of protein but cows do not bloat when fed these grasses.

Saponins. It has been suggested by several investigators (Lindahl et al., 1954; Olson, 1944; Quin, 1943) that saponins, naturally occurring plant glucosides, are contributing factors in the pathogenesis of ruminant bloat, especially legume bloat. Two different saponins have been found in alfalfa (Lindahl et al., 1954). Jacobson in 1919 fed a sheep 19 gm. of saponin and reported that the animal did not produce bloat. Lindahl et al. (1954) observed that maximum distention of the rumen occurred in 30 to 45 minutes with alfalfa saponin whereas alfalfa or ladino clover juice produced the same degree of distention in 10 to 15 minutes. They observed that the distention in both cases was due to retention of gas rather than stable foam. Henrici (1952) demonstrated that saponins of the legumes associated with zinc deficiency of the soil could produce pasture bloat in cattle. McGandlish (1937) incriminated saponins as being responsible for frothing of rumen ingesta and the production of bloat.

Thompson (1957) demonstrated that legumes contain comparatively larger amounts of saponin than do grasses and suggested that the saponins of legumes are involved in the etiology of bloat. Jackson and Shaw (1960) encountered a saponin of legumes which inhibits muscle respiration and consequently is associated with the occurrence of bloat in cattle. Potter and Kummerow (1954) demonstrated that soybeans contain similar saponins as alfalfa and, therefore, contribute to produce feedlot bloat. Weiss (1953) postulated that the presence of saponins in alfalfa is not the cause of bloat per se, but merely contributes toward the colloidal state of the ingesta which favors foaming.

Lipids. The consensus of various workers on prevention of bloat is that fats and oils are bloat preventive agents. Legumes contain both foaming and anti-foaming agents. Plant chloroplasts contain a very high concentration of lipids, up to 37 per cent of the dry weight depending upon the species.

(Chinball, 1939; Neish, 1939). Mangan *et al.* (1959) observed that rumen liquor (penicillin treated) had poor foam stability before and strong foam stability after centrifuging the chloroplasts. Treatment with penicillin destroyed the lipolytic bacteria and thus, the chloroplast lipids contained in the rumen liquor possessed anti-foaming activity. Hill *et al.* (1960) observed that the lipase activity of rumen fluid from animals grazing alfalfa was several times greater than that of animals on dry feeds. This suggests that the lipolytic activity of microorganisms destroys the anti-foaming property of alfalfa chloroplast lipids. Scott (1955) has demonstrated that much of the lipid of the chloroplast occurs as a cloud of globules surrounding the chloroplast and is attached to the parent body by transparent plasmodesmata. This structure is expected to give chloroplasts anti-foaming properties. Mangan (1958) observed that isolated chloroplasts had considerable anti-foaming properties, whereas, fat free chloroplasts, obtained with acetone and ether treatment, had no anti-foaming property and had the reverse effect on stabilizing foam.

Saliva. Ruminants (cattle) salivate approximately 15 gallons of saliva per day (Nichols, 1959). The rate of secretion of saliva depends on a number of factors such as psychic influences, feeding methods, rumination, rumino-reticular motility, and dietary composition. Mastication of feed during rumination enhances the flow and swallowing of even larger quantities of saliva. Coarse feeds stimulate reflex salivation while rapid eating of succulent feeds results in less salivation per quantity of feed consumed.

Bailey (1959) observed that fibrous feeds were eaten slower than less fibrous feeds and stimulated a larger flow of saliva per unit weight of feed consumed.

Balch (1958) demonstrated that the salivary secretion rates for hay,

concentrates, and grass were respectively 5 to 6 pounds, 7 to 12 pounds, and 3 to 5 pounds per 10 minutes.

Although it is generally accepted that saliva serves as a digestive aid because it is a lubricant, solvent, and medium of transport, other properties of saliva appear to be of equal importance in rumen metabolism. Saliva influences the numbers and kinds of microorganisms by providing an environment which is optimal for microbial activity (Reid and Huffman, 1949).

It has been suggested that rumino-reticular motility affects the secretion of saliva. In legume bloat, toxic materials, like saponins released from the ingesta, inhibit the rumino-reticular motility (Cole and Boda, 1960) and consequently reduce the rate of secretion of saliva. Denton (1956) reported that chopped green alfalfa reduced the output of parotid saliva compared with that produced by the addition of dry roughage.

There is appreciable difference in the components of saliva produced by different types of salivary glands (Johns, 1958). The flow of parotid saliva is continuous, contains a large quantity of bicarbonate but very little mucoprotein, and has a high buffering capacity. Submaxillary saliva is secreted while the animal is consuming feed. It has a low concentration of bicarbonate and low buffering power. This type of saliva contains a comparatively large amount of mucoprotein and is viscous. Residual saliva is secreted mainly by the sublingual salivary glands. It contains an intermediate amount of bicarbonate and mucoprotein.

Similar patterns of flow of saliva within an identical twin-set of cows but different patterns between twin-sets have been observed by Lyttelton (1960). He reported that the major component of bovine saliva is a mucoprotein containing sialic acid. Ruminant saliva is alkaline since it contains a considerable amount of bicarbonate and phosphate. These salts serve to buffer

acids produced by microbial fermentation in the rumen.

There appears to be a difference of opinion regarding the possible part played by saliva in the bloat syndrome. Johns (1954) suggested that part of the contribution of the animal to foam production could be due to saliva. The CO₂ produced by decarboxylation of mucoproteins was thought to be the main offender. Reid and Huffman (1949) suggested that the low surface tension of bovine saliva promoted formation of froth. In support of Johns hypothesis, Mangan (1958) found that when mixed bovine saliva was aerated, a stable foam was formed. Phillipson and Reid (1958) reported that intra-rumen gas pressure increased the salivary flow and aggravated the moderately bated animals. This was due to release of CO₂ from salivary bicarbonates and mucoproteins.

Weiss (1953) found that bloat in cattle occurred immediately after feeding succulent, leafy alfalfa, due to frothing of thick, viscid ruminal ingesta and on the other hand, when animals were fed stemmy alfalfa the ruminal ingesta was watery and bloat ceased. He concluded that the physical nature of stalky alfalfa stimulated the flow of saliva which consequently reduced the consistency of the ruminal contents. His hypothesis on etiology of legume bloat was ascribed to the diminished reflex salivary secretion which occurs when animals are fed succulent legumes.

The existence of the salivary reflex was demonstrated (Clark and Weiss, 1952) by mechanical stimulation of the mucous membrane of the forestomachs of ruminants.

Cole, Mead, and Regan (1943) found that the feeding of coarse hay before pasturing alfalfa could prevent bloat. This observation supports the hypothesis of Weiss.

Bartley (1957) observed that the consistency of ruminal ingesta varied between identical twin-sets (fistulated) pasturing on the same field. The

twins with watery ruminal ingesta were not so liable to bloat as those twins with a drier ruminal ingesta. He thought that the consistency of the ruminal ingesta was due to rate of secretion of saliva which was supposed to be an inherited characteristic in twin-sets. This supports the theory that the susceptibility to bloat is, in part, an inherited characteristic (Knapp *et al.*, 1943; Hancock, 1953; Johns, 1954; Lyttelton, 1960).

Bartley (1957) demonstrated that the ruminal ingesta incubated with saliva or mucilaginous extracts of linseed meal resulted in release of more gas than that incubated alone. He also observed that the incidence of bloat was reduced in cows fed linseed meal before pasturing. This led to the postulation that the mucin of saliva or linseed might have a bloat preventive effect.

Van Horn and Bartley (1959) observed that addition of saliva or mucin to frothing rumen contents resulted in escape of more gas than that of controls. They also reported that stable froth was formed when air was bubbled through a saponin solution but addition of saliva or mucin prevented the formation of a stable form.

Van Horn (1959) studied the effect of addition of animal mucin to fistulated twins pasturing on alfalfa and observed a marked difference in the foaming ability of the ruminal ingesta of treated and untreated twins.

Yadava (1960) tested the anti-foaming effect of saliva, linseed mucin, and mucins from animal sources *in vitro*. Addition of each of these reduced the formation of stable foam which occurred when alfalfa saponin solution was aerated.

Van Horn (1959) injected cows with 50 mg. and 75 mg. of atropine sulfate, an antisialagogue, subcutaneously to reduce the flow of saliva and test its effect on the production of bloat. He did not find any difference between treated and untreated cows during three days of study. In contrast to this,

Yadava (1960) reported that injection of 50 mg. atropine sulfate produced no clear cut difference in foaming on the first day of injection, but from the second day on there was significantly more foaming in the treated than in the untreated cows of the twin pairs. It is evident from the latter experiments that rate of secretion of saliva has a definite effect on the production of froth in the rumen.

In support of Van Horn's work Bartley and Yadava (1961) observed that bloat was prevented for four hours when animal mucins were introduced into the rumen of cattle grazing alfalfa pasture. The short period of effectiveness led to their concept that mucin is in someway destroyed or inactivated in the rumen. It was suspected that the agent responsible was of microbial origin.

Role of Microorganisms in the Production of Bloat

The role of microorganisms in ruminant nutrition has been under investigation for sometime. The large amount of gas produced in the rumen is due to the microbial fermentation. Changes in characteristics or numbers of rumen microflora occur from time to time and vary according to the diet. Recent research on rumen microbiology has indicated a relationship between rumen microflora (both fauna and flora) and bloat. Moreover, temporary prevention of bloat with antibiotic treatments further substantiate this hypothesis. The production of gas due to microbial fermentation may not be the only reason for formation of stable foam, but several other agents produced by these organisms are suggested to be involved. Frye et al. (1956) suggested that a waste product formed through bacterial metabolism might cause a paralysis of the ruminal musculature and produce a loss of sensitivity in the area where eructation is stimulated.

Hungate et al. (1955) suggested that slime produced by microorganisms may be associated with frothy bloat. Slimes retard the coalescence of minute bubbles which consequently become trapped by the ruminal contents to produce froth. Jacobson and Lindahl (1955) postulated that slime production in the rumen is a possible factor in the production of feedlot bloat.

Gutierrez et al. (1958) showed that facultatively anaerobic amylolytic streptococci increased greatly in numbers in the rumen as cattle started to bloat when fed a high grain, feedlot bloat producing ration. They presumed that slime production by microorganisms is involved in stable foam production. Gutierrez et al. (1958) also observed that the rumen bacteria could degrade alfalfa saponins with the concomitant production of slime. They isolated encapsulated lactic acid streptococci during the onset of feedlot bloat.

Rosen et al. (1956) found that succinic acid, malic acid, malonic acid, and citric acid possessed gas producing activity whereas, various other organic acids, amino acids, sugars, etc. had little or no gas producing activity. They suggested that the decarboxylation of the above substrates produced by microbial fermentation of alfalfa contributes to the formation of large amounts of gas. This gas produces froth when associated with saponins and colloids.

Mangan et al. (1959) suggested that penicillin prevented bloat by inhibiting the slime producing microorganisms or by reducing the rate of rumen fermentation and hence decreasing volatile fatty acid and gas production.

Johnson et al. (1960) suggested that the prevention of bloat with antibiotics is due to the development of resistant strains of bacteria or to the changes in the balance of the ruminal microflora.

Plant chloroplasts are believed to have anti-foaming properties. Oxford (1958) reported that the microorganisms ingest whole chloroplasts and suggested

that bacteria might play a part in bloat by removing anti-foaming agents from these plants.

The role of saliva and mucin as bloat preventive agents was discussed earlier. Since the effectiveness of both of these agents was of short duration it was conjectured that they might be degraded rapidly by the mucinolytic bacteria of the rumen (Fina *et al.*, 1961). The mucin splitting enzyme produced by Vibrio cholerae was first reported by Burnet and Stone (1947) and was called the Receptor Destroying Enzyme (RDE). Burnet (1949) described an enzyme in culture filtrate of Vibrio comma which depolymerized ovomucin and glandular mucins but had no action on hyaluronic acid containing mucins. Singh and Ahuja (1953) found mucinase activity in culture filtrates of noncholerae vibrios. Formal and Lowenthal (1956) described the mucinolytic activity of certain strains of Shigella flexneri. Williams and Powlen (1959) reported that strains of Staphylococcus aureus, Aerobacter cloacae, vegetative and spore cells of Bacillus subtilis and Bacillus cereus grew suboptimally on human parotid saliva as the sole source of nutrient. Lowenthal and Berman (1959) demonstrated the production of mucinase in the species Clostridium histolyticum, Cl. perfringens, Cl. sporogenes, and Cl. tertium. Bergamini (1956) demonstrated the enzymatic activity of a few strains of Actinomyces albus, Staphylococcus aureus, Bacterium prodigiosum, Bacillus anthracis, and a Coccobazillen not identified, upon epithelial mucin with the consequent decrease in viscosity of mucin. Hungate *et al.* (1955) observed mucilaginous material in cultures of a spore forming, actively cellulolytic rod which they isolated from the rumens of several cows.

Hay (1961) determined the mucinolytic activity of a few rumen organisms by growing them on Lord's carbon free media containing saliva as the sole source of organic nutrients. These organisms were then introduced into the

rumens of fistulated cows pasturing on alfalfa to determine the bloat provoking ability of mucinolytic bacteria. He observed that the animals inoculated with these mucinolytic bacteria developed severe bloat.

Bartley et al. (1961a) studied the incidence of bloat in fistulated twin pairs. They completely emptied the rumens and allowed the animals to graze alfalfa pasture. These animals developed bloat on the fourth day which was believed to be due to development of functional rumen flora from the feed during the first three days of grazing. They suggested that bloat is not the result of a simple physical breakdown of feed, since in these experiments, bloat did not occur on the second or third day. This supports the theory that there is a definite relationship between microorganisms and the production of bloat in cattle.

Feedlot Bloat

Smith et al. (1953) reported that frothy bloat could be produced by feeding 16 pounds of a grain mixture and 4 pounds of long alfalfa hay per day. They observed that equal amounts of grain mixture and hay would sustain the frothing condition once it had been initiated. Blake et al. (1955) induced bloat in cattle by feeding a ration composed of: ground corn, 60 per cent; soybean oil meal, 17 per cent; alfalfa meal, 20 per cent; and minerals, 3 per cent.

Lindahl et al. (1957) studied the effect of several diets on the production of feedlot bloat. These diets were: IA., 14 pounds of concentrate (78.3 per cent corn, 20.7 per cent soybean oil meal, and 1 per cent salt) and 4 pounds of No. 2 alfalfa hay per animal per day; IB, same as IA but 4 pounds of alfalfa meal replaced alfalfa hay; IC, same as IA except the salt contained

one part of a sulfated monoglyceride type detergent to four parts of salt; and 2A, 2B, 2C, same as above but barley was substituted for corn. Bloat was encountered on all rations and there was no significant difference among diets in the incidence of bloat. Animals varied in susceptibility to bloat and there was a relationship between the incidence of bloat and the length of time the animals had been maintained on the ration.

Elam and Davis (1959) produced feedlot bloat with a pelleted ration composed of 61 per cent barley, 16 per cent soybean meal, 22 per cent dehydrated alfalfa, and 1 per cent salt.

Wallace (1960) considered that in feedlot bloat, feed particles stabilize the foam bubbles by adhering to the air/water interface.

Jacobson et al. (1957) noticed that the number of encapsulated micro-organisms reached a high level in the rumen fluid of bated cows fed feedlot bloat rations. Jacobson et al. (1958) found that the molar concentration of total volatile fatty acids of rumen fluid of cows fed feedlot bloat rations was high. They suggested that presence of high energy feeds and changes in the microbial flora of the rumen were responsible for this change. They observed that the rumen liquor did not contain reducing substances but lactic acid was invariably found in the rumen sample collected 4.5 hours after feeding a bloat producing diet. This is apparently due to dissimilation of glucose and cellobiose by rumen bacteria.

Lindahl et al. (1957) observed that the bloat produced with feedlot rations was of the frothy type but a varying amount of free gas was always present.

EXPERIMENTAL METHODS

Experimental Design

In preliminary trials a ration was developed which would produce a feedlot type bloat in a majority of animals. This ration was then fed to fistulated identical-twin cows. After frothy bloat developed, the minimum amount of coarse, long alfalfa hay needed to prevent bloat was determined. After bloat ceased, five experiments were conducted to determine if large quantities of rumen mucinolytic bacteria introduced into the rumens of these animals could provoke bloat. These studies were repeated using nonfistulated dry cows (experiment VI). Nonfistulated identical-twin heifers (experiments VII and VIII) were used to determine if rumen mucinolytic bacteria could produce bloat when animals are fed normal rations of silage, hay, and cottonseed meal. Experiments X, XI, XII, and XIII were conducted to determine the effect of mucinolytic or normucinolytic bacteria of rumen or nonrumen origin on the production of feedlot bloat.

In all experiments one member of each twin pair (or member of each group when twins were not used) was inoculated with bacteria and the other served as a control. Each experiment was replicated with the treatments being reversed.

Animals Used

Three pairs of identical-twin dry cows were used in Experiments I through V. Each animal had a permanent rumen fistula fitted with a plastic cannula and a screw cap. These three pairs consisted of: one pair of 5 year old Jersey cows (81-82), one pair of 5 year old Guernsey cows (22-23), and one pair of

5 year old Brown Swiss cows (31-32).

Four nonfistulated dry cows (275 B, 280 B, 158 B, and 466 C) were used in Experiment VI. Two Hereford heifers (A-B) and one crossbred Hereford-Holstein (C) unfistulated heifer were used in Experiment VII. These three heifers were about three years old.

Three pairs of nonfistulated identical-twin heifers were used in Experiments VIII, XIII, and XIII to determine if they would react similarly to fistulated animals. These comprised: one pair of two year old crossbred Hereford-Holstein (91-92), one pair of two year old Jerseys (78-79), and one pair of three year old Guernseys (3C-4C).

All animals were housed in the same room and had drinking water and salt available at all times. Straw was used as bedding in the beginning but as pieces of straw were detected in the rumen ingesta, wood shavings were employed as bedding during the remainder of the study.

Feeding Methods

All animals were individually fed twice daily. The feedlot bloat producing ration fed daily to each of the fistulated animals consisted of: dehydrated alfalfa pellets, 4 pounds; ground corn, 12 pounds; and soybean oil meal, 2 pounds. The nonfistulated dry cows were also fed the same amounts of the above feeds.

The nonfistulated heifers (91, 92, 78, and 79) were each fed daily: dehydrated alfalfa pellets, 2 pounds; ground corn, 6 pounds; and soybean oil meal, 2 pounds. The nonfistulated Guernsey heifers (3C and 4C) were each fed 2 pounds of additional ground corn per day.

Two Hereford heifers and one crossbred Hereford-Holstein heifer were each

fed daily the following ration: 6 pounds long hay, 2.5 pounds cottonseed oil meal, and corn silage ad libitum. This was the normal ration fed to the heifers in dry lot.

The animals were maintained on the bloat producing diet until bloat was induced (usually four weeks). Hay was then fed to stop bloat. Since 4 pounds of coarse, long alfalfa hay would prevent most of the bloat encountered, this level of hay feeding was used during the major portion of the experimental period. Diarrhea and inappetence were sometimes encountered. These conditions were treated by feeding more hay and less grain.

Rating of the Degree of Bloat

The degree of bloat displayed by both the treated and control animals was rated according to the scale shown in Table 1. Nonfistulated animals were rated according to the scale shown in Table 2. The degree of bloat was recorded twice daily (morning and evening). In some instances the animals were checked for bloat 2, 4, and 6 hours after rumen inoculation. The animals were always scored by the author.

Microorganisms Used

The microorganisms used were: (1) mucinolytic bacteria H isolated from the rumen by Hay (1961) and (2) known mucinolytic and nonmucinolytic organisms of rumen and nonrumen origin:

1. Aerobacter aerogenes
2. Escherichia coli
3. Proteus vulgaris

4. Salmonella pullorum
5. Sarcina lutea
6. Staphylococcus albus
7. Staphylococcus aureus
8. Streptococcus faecalis
9. Lactobacillus acidophilus
10. L. buchneri
11. L. casei
12. L. plantarum.

Table 1. Scale used in rating frothy bloat in fistulated animals.

Score:	Degree of frothing	Degree of abdominal distention		Other abnormalities
		Left side	Right side	
0	None	None	None	None
1	Slight	None	None	None
2	Definite	None	None	None
3	Frothy rumen ingesta gushes out several feet when fistula cap is removed	Slight to moderate	None	None
4	" "	Definite, drum like	Slight	Restless
5	" "	Extremely dis- tended, left hip hidden, skin light	Extremely distended	Frequent defeca- tion, urination, muscular incoord- ination, protrud- ing anus, respira- tory distress

Table 2. Scale used in rating frothy bloat in nonfistulated animals.¹

Score :	Description
0	No bloat -- No distention in left paralumbar fossa.
1	Slight ---- Slight distention in left paralumbar fossa; "puffy".
2	Mild ----- Marked distention in left paralumbar fossa; well rounded out between hip and rib on left side; little or no distention on right side.
3	Moderate -- Well rounded out on left side, drumlike; full on right side; restless.
4	Severe ---- Both sides badly distended; left hip nearly hidden; skin tight; defecation; urination; inco-ordination; protruding anus; mild respiratory distress.
5	Terminal -- Extreme abdominal distention; severe respiratory distress; cyanosis; prostration; death unless treated.

¹Johnson, et al., 1958.

Identification of Mucinolytic Bacteria

The microorganisms selected for study were cultivated in Lord's carbon free agar medium to which 25 per cent Seitz filtered saliva was added. Streak plates were prepared and incubated at 100° F. Period of incubation varied from 24 to 48 hours. Those organisms which grew on the surface of this medium were characterized as being mucinolytic. The formation of colonies was the criterion used for determining growth.

Collection of Saliva

A cow was injected with 3 ml. of Lentin (carbachol) subcutaneously in the neck region and the saliva which dripped from the mouth was collected in a clean Erlenmeyer flask to which a funnel was fitted. Saliva collection started within 2 minutes of injection and usually one liter was collected within one hour. The nasal discharge was discarded by frequent wiping of the nostrils of the cow. The saliva collected was sterilized through a Seitz filter and stored in a sterile flask in the refrigerator.

Preparation of Carbon Free Medium

Lord's (1959) carbon free agar medium was prepared with the following ingredients:

Dipotassium phosphate (K_2HPO_4)	1. gm.
Magnesium sulfate ($MgSO_4$)	0.5 gm.
Sodium chloride ($NaCl$)	0.01 gm.
Ferrous sulfate ($FeSO_4 \cdot 4 H_2O$)	0.01 gm.
Manganous sulfate ($MnSO_4 \cdot 4 H_2O$)	0.01 gm.
Agar agar	20. gm.

The ingredients were weighed and dissolved in 750 ml. of distilled water by boiling. The mixture was autoclaved for 15 minutes at 248° F. (15 pounds pressure). The sterilized medium was cooled down to 110° F. The Seitz filtered saliva was warmed in water bath to 110° F. and 250 ml. of saliva was added to 750 ml. of medium. The contents were mixed slowly to prevent foam formation and plates were made in sterile Petri dishes.

Preparation of Cultures of Microorganisms for Animal Inoculation

Standard nutrient broth was prepared by dissolving 5 gm. Bacto-peptone and 3 gm. beef extract in a liter of tap water. The medium was sterilized in the autoclave at 248° F. under 15 pounds steam pressure for 30 minutes. Immediately after the medium cooled it was inoculated with the test organism and incubated at 100° F. for 36 to 48 hours. In the first few experiments the entire medium with bacterial cells was introduced into the rumens of the experimental animals. In later experiments the culture medium was centrifuged with one of the following centrifuges: Serval super centrifuge (20,000 RPM); Sharples super centrifuge (20,000 RPM); and International centrifuge No. 1 (2,000 RPM). The sediment containing the bacterial cells was suspended in saline (cells centrifuged out of one gallon of culture medium were suspended in one liter of physiological saline) before use.

Lactobacilli were grown in yeast extract medium containing 10 gm. dextrose, 5 gm. yeast extract, 50 ml. strained tomato juice, and water to make one liter.

Inoculation of Cows with Bacteria

After feedlot bloat was prevented by feeding 4 pounds of long hay, one of each pair of twins was inoculated (rumen) with the culture of one species of organism. The saline-cell suspension was placed in the rumens of the fistulated cows or introduced into the reticulo-rumen of the nonfistulated heifers with a stomach tube and pump.

In some experiments rumen inoculation was made only once and in other experiments the rumen was inoculated every third day to enhance the

establishment of the introduced microorganism.

RESULTS

Isolation of Bacteria Capable of Utilizing Bovine Saliva

The ability of one unidentified rumen organism H isolated by Hay (1961) and 12 species of known organisms to grow on the medium containing saliva as the sole carbon and nitrogen source was determined. These results are reported in Table 3. It was observed that the unidentified rumen organism H was mucinolytic in agreement with results obtained by Hay, 1961. Later it was confirmed (Fina, unpublished) that organism H is not an individual species but is a mixed culture. Efforts have been made by the Department of Bacteriology, Kansas State University, to isolate and identify these microorganisms.

Staphylococcus albus, Sarcina lutea, and Escherichia coli grew well on this medium and were classified as mucinolytic. Staphylococcus aureus and Proteus vulgaris did not grow well and it is doubtful whether or not they are mucinolytic. Aerobacter aerogenes, Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus buchneri, Lactobacillus plantarum, Streptococcus faecalis, and Salmonella pullorum did not grow on this medium and were designated as being nonmucinolytic for the present, pending further investigation.

The lactobacilli group of organisms need specific vitamins and amino acids for optimum growth. Under the conditions of this experiment, no extra vitamin or protein, except mucin, was available and hence it cannot be concluded that they are nonmucinolytic even though none of the species of this genus grew on this medium.

Table 3. Growth of microorganisms on Lord's carbon free medium containing saliva.

Microorganisms	Growth after 24 hours of incubation	Growth after 48 hours of incubation
H (rumen bacteria)	+	*
Replicate of above	+	*
<u>Staphylococcus albus</u>	+	*
Replicate of above	+	*
<u>Sarcina lutea</u>	+	+
Replicate of above	+	+
<u>Escherichia coli</u>	+	+
Replicate of above	+	+
<u>Staphylococcus aureus</u>	±	±
<u>Proteus vulgaris</u>	±	±
<u>Aerobacter aerogenes</u>	-	-
<u>Streptococcus faecalis</u>	-	-
<u>Salmonella pulorum</u>	-	-
<u>Lactobacillus casei</u>	-	-
<u>Lactobacillus acidophilus</u>	-	-
<u>Lactobacillus buchneri</u>	-	-
<u>Lactobacillus plantarum</u>	-	-

- No growth

⊕ Positive growth

± Suspicious (appearance of very few colonies)

* Incubated for 24 hours only

Production of Feedlot Bloat

Cows 81, 82, 31, 32, 22, and 23 were available for feedlot bloat studies on December 9, 1960. Previous to this, these cows were on another experiment where a ground hay ration was fed for a few weeks. All the cows were then changed over to the feedlot bloat producing ration. No hay was fed. Before feeding this ration, all cows were checked for the presence of bloat but none had frothy ruminal contents. On the second day of feeding, the feedlot bloat producing ration all cows bloat and two had diarrhea. After the third day 2 pounds of hay was fed twice daily to each cow to prevent bloat. After four days the ruminal ingesta of all the cows ceased frothing. Rumen inoculations with cultures of microorganisms were conducted after bloat ceased. These results are presented later.

All three pairs of unfistulated heifers developed feedlot bloat when fed the feedlot bloat provoking ration. In almost all instances, bloat appeared about 25 days after the animals had first been fed the bloat producing ration.

Feedlot Bloat Following Inoculation with Mucinolytic Rumen Organism-H

In Experiment I (Table 4) the control cows developed bloat, although to a lesser extent than the inoculated animals. Undoubtedly nutrient broth per se is capable of producing frothy bloat. In order to remove the bloat producing effects of nutrient broth from the controls, the test organisms were suspended in one liter of physiological saline. It is apparent (Table 5) that saline alone had no effect on bloat.

In Experiment II (Table 5) definite bloat occurred in all treated animals

within 12 to 24 hours after inoculation, while no change occurred in the degree of bloat in the control animals. The quantity of hay fed did not completely prevent bloat in twin pair 31-32. However, after inoculation the treated animal bloated more severely than its twin control.

In Experiment III (Table 6) the bloat index of the treated animal was significantly higher than that of its control.

In Experiment IV (Table 7) the quantity of hay fed did not completely prevent bloat in two control animals (82 and 32). This was probably due to a carry-over effect from the two previous experiments. However, after inoculation the treated animals bloated more severely than their twin controls.

In Experiment V (Table 8) the animals were reinoculated during the trial in an attempt to establish rumen organism H and provoke bloat continuously. This experiment was conducted for 31 days and four inoculations were made. Before this experiment commenced almost all bloat was brought under control by feeding long hay. It is apparent from the data (Table 8) that bloat was maintained in the treated animals for 30 days by periodic inoculation with the mucinolytic organism. Bloat persisted in the treated heifers at the same degree until 12 days after the last inoculation when this experiment ended. This suggests that establishment of certain mucinolytic organisms in the rumens of cattle may be an important factor in inducing feedlot bloat.

Production of Bloat in Nonfistulated Dairy Cows and Heifers Following Inoculation with Mucinolytic Rumen Bacteria H

Four dry dairy cows were inoculated with rumen organism H initially in Experiment VI and reinoculated thrice with the same organism during a 34 day trial (Table 9). The inoculated cows did not show definite signs of bloat

after the first inoculation, but developed bloat after the second inoculation. Bloat subsided four days after the second inoculation. Again following the third inoculation, the bloat index was increased over the control animals. After 14 days the control cows developed bloat of the same degree as the treated cows. Bloat in the controls persisted until the end of the experiment. These results indicate that the feedlot ration itself, if fed long enough, may alter the rumen flora and produce continuous bloat in uninoculated cows.

In Experiment VII (Table 10) three beef heifers were used to determine whether the mucinolytic rumen organism H could produce feedlot bloat when animals are fed a high roughage ration. All three heifers were drenched with the saline suspension of organism H. No sign of bloat was observed in any of the heifers although repeated inoculations were made. Heifer No. C was inoculated with the organism grown in three gallons of nutrient broth for 48 hours, but no bloat occurred. These results suggest that these mucinolytic bacteria may not be capable of utilizing the larger amounts of saliva which should be secreted by these heifers when fed high roughage rations. Also it might be that the presence of large amounts of roughage type microorganisms in the rumen prevented the establishment of sufficient quantities of bloat producing mucinolytic bacteria.

Experiment VIII was conducted with a pair of young identical-twin non-fistulated crossbred Hereford-Holstein heifers. The inoculated heifer (No. 91) produced bloat (Table 11) within 12 hours after inoculation with rumen organism H and continued bloating for 13 days. The control heifer (No. 92) had very slight bloat only for three days. This result agrees with that of Experiment VI except that this pair was not maintained on a high grain ration for an extensive period of time.

Effect of Mucinolytic or Nonmucinolytic Bacteria of Rumen or Nonrumen Origin on the Production of Feedlot Bloat

From the data presented in Experiments IX, X, XI, XII, and XIII (Tables 12, 13, 14, 15, and 16), it is apparent that under the conditions of these experiments mucinolytic organisms, whether or not they are of rumen origin, will provoke a greater degree of bloat than nonmucinolytic bacteria. With three exceptions, all animals receiving organisms exhibiting mucinolytic activity, bloated more severely than their uninoculated controls or animals receiving organisms exhibiting no mucinolytic activity. Details of the results encountered with individual organisms are discussed below.

Staphylococcus albus. In Experiment IX and X (Tables 12 and 13) S. albus produced a high degree of bloat in fistulated cows. This organism was shown to be mucinolytic (Table 3). In Experiment IX, the bloat continued for 15 days after which it subsided. In Experiment X the bloat index was 4 and persisted for the duration of the experiment (7 days).

Escherichia coli. In Experiment IX (Table 12) cow No. 32 had a bloat index of 2 before being inoculated with E. coli. Within 12 hours after inoculation the bloat index increased to 3 and persisted for only 2 days. These results suggest that E. coli which is mucinolytic and a member of the rumen flora may utilize saliva to some extent and, therefore, produces bloat.

Aerobacter aerogenes. In Experiment IX (Table 12) this organism did not provoke bloat, but in Experiment X a slight foam was observed for a few days. The organism is nonmucinolytic and the results obtained suggest that it is unable to produce bloat.

Sarcina lutea. The Sarcina group of organisms has been isolated from the rumen (Baker *et al.*, 1950). They were found to be mucinolytic. In Experiment

IX (Table 14) cow No. 23 had a bloat index of 3 after inoculation. This degree of bloat persisted for 5 days after which it subsided to a degree of 1. In Experiment X (Table 13) cow No. 23 had a bloat index of 4 which persisted for 7 days (end of the experiment).

Streptococcus faecalis. As shown in Experiment X (Table 13) cow No. 31 exhibited a bloat index of 3 on the third and fourth days after inoculation with S. faecalis and then maintained an index of 2 for four days (duration of experiment).

Proteus vulgaris. This organism is mucinolytic and produced a bloat index of 3 within 12 hours of inoculation in cow No. 32 in Experiment XI (Table 14). This degree of bloat persisted for five days of study.

Salmonella pullorum. This organism is nonmucinolytic and did not produce bloat after rumen inoculation in Experiment XI (Table 14).

Lactobacilli. A number of organisms of this group has been isolated from the rumen contents (Jensen *et al.*, 1956; Perry and Briggs, 1957). In this study four species of Lactobacilli: L. buchneri, L. plantarum, L. casei, and L. acidophilus were used. Of these four species, L. buchneri produced a high degree of bloat in both experiments X and XI (Table 13 and 14). L. plantarum also produced a high degree of bloat on the third day after inoculation but maintained this degree of bloat for only four more days. L. acidophilus produced only slight foam (degree of 1) in cow No. 81. This degree of bloat persisted for four days. L. casei was unable to produce bloat in cow No. 22. It was found to be nonmucinolytic (Table 3).

In Experiments XIII and XIV, nonfistulated heifers were employed for testing the bloat provoking ability of Staphylococcus albus and Lactobacillus buchneri. In Experiment XII (Table 15) S. albus produced a bloat index of 2, whereas the control animal did not bloat. In Experiment XIII (Table 16)

slight bloat occurred in inoculated heifers (91 and 4C) and no bloat occurred in the control heifers (92 and 3C).

Two exceptions to the rule that nonmucinolytic organisms do not provoke bloat are Lactobacillus buchneri and Lactobacillus plantarum. It is possible that these organisms may be mucinolytic in reality but failed to exhibit mucinolytic activity in the in vitro test because they need specific amino acids and vitamins which were not provided by Lord's carbon free medium. Thus, the in vitro test for mucinolysis used in this study may not be confirmatory for all organisms.

Table 4. Feedlot bloat following inoculation¹ of fistulated identical-twin cows with mucoinolytic rumen organism H.

Experiment I

		Maximum bloat index					
		Date					
		before	inoculation	Dates after inoculation			
Twin		inoculation				12-9-60 12-10-60 12-11-60 12-12-60 12-13-60 12-14-60 12-15-60 12-16-60	
pairs: Treatment:							
61	Control	0	3	3	2	2	0
62	Inoculated	0	4	4	4	4	3
31	Control	0	3	3	2	1	0
32	Inoculated	0	4	4	4	3	3
23	Control	0	3	2	2	0	0
22	Inoculated	0	4	4	3	2	1

¹Treated animal received organism H in 1 gallon nutrient broth. Control received only 1 gallon nutrient broth.

Table 5. Feedlot bloat following inoculation¹ of fistulated identical-twin cows with mucinolytic rumen organism H.

Experiment II

Twin pairs:	Treatment	Date before inoculation		Maximum bloat index			
		inoculation		Dates after inoculation			
		12-24-60	12-25-60	12-26-60	12-27-60	12-28-60	12-29-60
81	Inoculated	0		4	3	3	2
82	Control	0	0	0	0	0	0
31	Inoculated	2		4	4	3	2
32	Control	2	2	2	2	0	0
23	Inoculated	0	3	3	2	0	0
22	Control	0	0	0	0	0	0

¹Centrifuged bacterial cells suspended in saline.

Table 6. Feedlot bloat following inoculation¹ of fistulated identical-twin cows with mucoinolytic rumen organism H.

		Experiment III											
		Maximum bloat index											
		Date											
		before inoculation		Dates after inoculation, January 1961									
Twin pairs	Treatment	12-31-60		1	2	3	4	5	6	7	8	9	10
81	Control	2	1	0	0	1	0	0	0	0	0	0	0
82	Inoculated	0	4	4	4	4	5	2	3	3	3	3	2

¹Centrifuged bacterial cells suspended in saline.

Table 7. Feedlot bloat following inoculation¹ of fistulated identical-twin cows with monoclonal Tissue organism H.

Experiment IV

Twin pairs	Treatment	Maximum bloat index		
		Date		Dates after inoculation
		before	after	
81	Inoculated	0	4	3
82	Control	2	2	3
31	Inoculated	0	3	4
32	Control	2	3	2
22	Inoculated	0	3	3
23	Control	0	0	0

¹ Centrifuged bacterial cells suspended in saline.

Table 8. Feedlot bloat following inoculation and reinoculation of fistulated identical-twin cows with macrolytic rumen organism H.

Experiment V

		Maximum bloat index															
		Date		before inoculation		Dates after inoculation											
Twin	Treatment	2-5-61	2-4-61	2-5-61	2-6-61	2-7-61	2-8-61	2-9-61	2-10-61	2-11-61	2-12-61	2-13-61	2-14-61	2-15-61	2-16-61	2-17-61	2-18-61
81	Control	1	1	0	0	0	2	1	1	1	1	1	1	1	1	0	0
82	Inoculated	0	4	4	4	3	4	4	3	3	4	4	4	4	3	42	32
31	Control	0	0	0	0	1	1	1	1	0	1	1	0	0	0	0	0
32	Inoculated	1	4	4	4	3	3	3	3	3	4	3	3	32	3	22	
22	Inoculated	0	2	2	2	3	3	3	23	0	1	2	2	32	3	42	
23	Control	0	0	0	0	0	0	0	1	1	2	2	1	0	0	0	0
		2-19-61 2-20-61 2-21-61 2-22-61 2-23-61 2-24-61 2-25-61 2-26-61 2-27-61 2-28-61 2-29-61 2-30-61 2-31-61 2-32-61 2-33-61 2-34-61 2-35-61															
81	Control	1	0	0	0 ²	1	0	0	0	1	2	1	1	0	1	1	1
32	Inoculated	0	4	4	4 ²	4	4	4	4	2	2	2	4	4	4	4	4
31	Control	0	0	0	1	1	0	0	1	1	1	1	1	2	2	2	2
32	Inoculated	1	4	4	4 ²	4	4	4	4	4	4	4	4	4	4	4	4
22	Inoculated	0	4	4	4 ²	4	4	4	4	3	3	3	3	3	3	3	3
23	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

¹Centrifuged bacterial cells suspended in saline.

²Reinoculations.

³Evacuated the rumen contents.

Table 9. Feedlot bloat following inoculation¹ and reinoculation of dry dairy cows with mucinolytic rumen organism H.

Experiment VI

		Maximum bloat index												
		Date		After inoculation										After reinoculation
		before		12-3-61; 1-12-3-13-3-14-3-15-3-16-3-17-3-18-3-19-3-20-3-21-3-22-3-23-3-24-3-25-3-26-3-27-3-28										
Animals: Treatment 12-3-61; 1-12-3-13-3-14-3-15-3-16-3-17-3-18-3-19-3-20-3-21-3-22-3-23-3-24-3-25-3-26-3-27-3-28														
275	Inoculated	0	2	0	0	0	3 ²	3	2	0	3 ²	3	3	3
280	Control	0	0	0	0	0	0	0	0	0	0	0	0	3
158	Inoculated	0	2	0	0	0	3 ²	3	2	0	3 ²	3	3	3
466	Control	0	0	0	0	0	0	0	0	0	0	0	0	3
3-29-3-30-3-31-4-1 14-2 14-3 14-4 14-5 14-6 14-7 14-8 14-9 14-10 14-11 14-12 14-13 14-14														
275	Inoculated	0	3	3	3	3	3	3	3	3	3	3	3	3
280	Control	0	3	3	3	3	3	3	3	4	4	4	4	4
158	Inoculated	0	3	3	3	3	3	3	3	3	3	3	3	3
466	Control	0	3	3	3	3	3	3	3	3	3	3	3	3

¹ Centrifuged bacterial cells suspended in saline.

² Re inoculations.

³ Controls inoculated.

Table 10. Feedlot bloat following inoculation¹ of nonfistulated beef heifers with rumen organism H.

Experiment VII

Animals	Treatment:	Maximum bloat index											
		Date		Inoculation 1961									
		before	inoculation	12-26	12-27	12-28	1-1	1-2	1-3	1-4	1-5	1-6	1-7
Hereford A	Inoculated	0	0	0	0	0	0	0	0	0	0	0	0
Hereford B	Inoculated	0	0	0	0	0	0	0	0	0	0	0	0
Hereford-Holstein C	Inoculated	0	0	0	0	0	0	0	0 ²	0	0	0	0

¹Centrifuged bacterial cells suspended in saline.

²Inoculum size was increased three times.

Table 11. Feedlot bloat following inoculation¹ of nonfastulated identical-twin heifers with mucinolytic rumen Organism H.

Experiment VIII

Twin pair: Treatment:	Date		Maximum bloat index						
	before inoculation	inoculation	Dates after inoculation 1961 6-22-61 6-23-61 6-24-61-25-61-26-6-27-6-28-6-29-6-30-7-1 7-2 7-3 7-4 7-5 7-6						
91 Inoculated	0	3	2	32	2	2	3	2	2
92 Control	0	0	1	1	1	0	0	0	0

¹ Centrifuged bacterial cells suspended in saline.

² Reinoculation

Table 12. Feedlot bloat following inoculation¹ of fistulated identical-twin cows with mucinolytic and non-mucinolytic organisms of rumen and nonrumen origin.

Experiment IX

Twin	Treatment ¹	Maximum bloat index												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Dates after inoculation, January 1961														
81	Control	2	1	0	0	0	1	0	0	0	0	0	0	0
31	<u>Staphylo-</u> <u>occus</u> <u>albus</u>	Pos	1	3	3	4	4	3	2	3	4	4	4	3
32	<u>Escherichia</u> <u>coli</u>	Pos	2	3	3	0	0	1	0	0	0	0	0	0
22	<u>Aerobacter</u> <u>serogenes</u>	Neg	0	0	0	0	0	0	0	0	0	0	0	0
23	<u>Sarcina</u> <u>lutea</u>	Pos	3	3	3	3	2	1	1	1	1	0	0	0

¹ Centrifuged bacterial cells suspended in saline.

Table 13. Feedlot bloat following inoculation¹ of fistulated identical-twin cows with mucinolytic and nonmucinolytic bacteria of rumen or nonrumen origin.

Experiment X											
Twin pairs:	Treatment	Date of inoculation:	Date before inoculation:	Maximum bloat index							
				4-30-61	5-1-61	5-2-61	5-3-61	5-4-61	5-5-61	5-6-61	
81	Aerobacter aerogenes	Neg		0	0	1	1	0	1	1	1
82	Staphylococcus albus	Pos	1		4	4	4	4	4	4	4
31	Streptococcus faecalis	Neg	0	2	3	3	2	2	2	2	2
32	Lactobacillus buchneri	Neg	0	4	4	4	4	4	4	3	
22	Lactobacillus casei	Neg	0	0	0	0	0	0	0	0	
23	Sarcina lutea	Pos	0	4	4	4	4	4	4	4	

¹Centrifuged bacterial cells suspended in saline.

Table 14. Feedlot bloat following inoculation¹ of fistulated identical-twin cows with mucinolytic and nonmucinolytic bacteria of rumen or nonrumen origin.

Experiment XI						
Twin pairs	Treatment activity	Inoculation date	Maximum bloat index			
			before inoculation	after inoculation		
				5-15-61	5-15-61	5-16-61
81	Lactobacillus acidophilus	Neg	0	0	1	1
82	Lactobacillus buchneri	Neg	2	4	4	4
31	Lactobacillus plantarum	Neg	2	4	3	3
32	Proteus vulgaris	Neg	0	3	3	3
23	Salmonella pullorum	Neg	0	0	0	0

¹Centrifuged bacterial cells suspended in saline.

Table 15. Feedlot bloat following inoculation¹ and reinoculation of nonfistulated identical-twin heifers with Staphylococcus albus.

		Experiment XII									
		Maximum bloat index									
		Date									
Twin pairs:	Treatment activity ²	Mucin- before olytic inocu- lation	Pos	0	2	22	2	2	22	2	2
3C	<u>Staphylococcus</u> <u>albus</u>										
4C	Control		0	0	0	0	0	0	0	0	0

¹Centrifuged bacterial cells suspended in saline.

²Reinoculations.

Table 16. Feedlot bloat following inoculation¹ of nonfistulated identical-twin heifers with Lactobacillus buchneri². Experiment XIII

Twin	Inoculation ¹	Maximum bloat index		Dates after inoculation
		Date	before	
		before	before	
91	Inoculated	0	2	1
92	Control	0	0	0
3C	Control	0	1	0
4C	Inoculated	0	2	2

pairs/treatment: 7-8-61; 7-9-61; 7-10-61; 7-11-61; 7-12-61

¹ Centrifuged bacterial cells suspended in saline.

² Nonmucinolytic.

DISCUSSION

From the earlier studies (Bartley, 1957; Bartley *et al.*, 1961; Bartley and Yadava, 1961) conducted at the Kansas station, it was postulated that mucin in saliva might be the antifoaming agent necessary to prevent bloat resulting from the consumption of lush alfalfa. Animal mucins introduced into the rumen of cattle grazing alfalfa prevented bloat for four hours only.

The results reported here suggest that the transitory bloat preventive effect of saliva is due to degradation or destruction of salivary mucin by mucinolytic organisms present or developed in the rumen before bloat occurs. With the exception of two species of nonmucinolytic organisms (Lactobacilli), all mucinolytic organisms tested induced bloat.

The results obtained with nonfistulated heifers with mucinolytic organisms, both of rumen and nonrumen origin, agree with those obtained with fistulated cows. However, the degree of bloat produced in the nonfistulated animals was lower in intensity than that occurring in the fistulated animals. This slight difference may be due to differences in environmental conditions prevailing in the rumens of fistulated and nonfistulated cows.

Mucinolytic rumen organism H, even in large amounts, could not induce bloat in heifers which were fed high roughage rations. Repeated inoculations (drenching) did not produce bloat. This is probably due to the inability of these bacteria to establish large enough concentrations in the rumens of non-fistulated heifers to overcome the activities of roughage type microorganisms. Also it is possible that the feeding of coarse hay and silage resulted in the secretion of large amounts of saliva which could not be degraded rapidly enough by the mucinolytic organisms present.

The role of antibiotics as curative and preventive agents in bloat has

been demonstrated by several workers (Barrentine *et al.*, 1956; Brown *et al.*, 1958; Van Horn *et al.*, 1960). This indicates a microbial role in the bloat syndrome. Microorganisms may be involved in bloat due to their mucinolytic activity, slime producing ability, or some other factors.

It may be concluded from the results reported here that mucinolytic micro-organisms may play an important role in the production of bloat. These results also support the theory that diminished salivary secretion and/or the destruction of mucin of saliva is conducive to the production of bloat.

It is obvious that bloat is a complex mechanism which embraces several factors as to its etiology and physiology. A number of hypotheses have been suggested for the cause of bloat. It has been established that the development of foam in the rumen is the main cause of bloat. Production of large amounts of gas is a normal phenomenon of the rumen and is associated with microbial fermentation. This gas is expelled by eructation. In the presence of foaming agents either from feed or animal origin, the gas becomes trapped in a stable foam which the animal is not able to eructate. Accumulation of increased amounts of stable foam in the reticulo-rumen results in bloat.

The foaming agents existing in plants include saponins, proteins, and pectins. Production of slimes from carbohydrates by certain rumen bacteria has also been incriminated. Inhibition of secretion of a normal amount of saliva into the rumen is an important factor in the etiology of both legume and feedlot bloat. It is clear that bloat is prevalent when cattle graze succulent legume pasture or are fed high concentrate-low roughage rations. In both these cases, the quantity of saliva secreted is diminished.

Lindahl *et al.* (1957) produced feedlot bloat in cattle after 42 days of feeding a high concentrate ration. In the study reported here, feedlot bloat was produced in four weeks. These results lead to the supposition that the

microbial environment of the rumen changes gradually following the change to certain rations and after a period of time certain groups of bloat producing organisms become established in the rumen and provoke bloat. Furthermore, Bartley et al. (1961) observed that when fistulated identical-twin cows with empty rumens were pastured on alfalfa, bloat did not occur until the fourth day, thus suggesting that bloat depends on establishing a certain concentration of microorganisms in the rumen. It is possible that changes in rations produce shifts in the ratios of rumen volatile fatty acids and pH, and that this change in the environment of the rumen affects the type and concentration of particular organisms in the rumen.

A lack of coarse roughage in the feed is suggested as being a factor in the incidence of bloat (Weiss, 1953; Cole et al., 1955). This lack leads to a diminished salivary flow and consequently an insufficiency of the natural anti-foaming factor mucin (Bartley, 1957). In support of the bloat preventing effect of coarse roughage, it was observed that three pairs of fistulated identical-twin cows which were previously fed a ground hay ration bloated within 24 hours of feeding the high grain bloat provoking ration. However, cows fed high roughage rations previous to feeding the high grain ration took about 4 weeks to develop bloat. It is evident from this that ground hay increased the bloating potential possibly by changing the types of rumen flora, thus making it easier for a high grain ration to trigger the production of bloat.

It may be concluded that mucinolytic organisms are not the only cause of bloat but they are intimately associated with bloat and are at least one factor in the bloat complex.

SUMMARY

Foaming of the rumen contents due to the presence of foaming agents in feeds and to a diminished salivary secretion has been recognized as a major factor in the etiology of legume and feedlot bloat. Since the effectiveness of saliva as an antifoaming agent in bloat was found to last only a few hours, it was conjectured that the natural antifoaming properties of saliva might be destroyed in the rumen by mucinolytic bacteria, thus permitting bloat to ensue. Hence studies were initiated to determine if mucinolytic organisms could provoke bloat in cattle fed a feedlot ration.

Three pairs of fistulated identical-twin cows, three pairs of nonfistulated identical-twin heifers, four dry dairy cows, and three beef heifers were used in this study.

One rumen mucinolytic organism (designated as organism H) was used in the first seven experiments. Several other organisms, both of rumen and nonrumen origin, were tested for mucinolytic activity. These organisms were used in later experiments.

The animals were maintained on a bloat provoking ration until bloat was induced. The minimum amount of long alfalfa hay needed to prevent bloat was fed. After bloat ceased, one member of each identical-twin pair was inoculated with one species of organism grown in nutrient broth for 36 to 48 hours. The other member of the twin pair served as a control.

In seven experiments cows fed the feedlot bloat ration with hay and inoculated with mucinolytic rumen organism H bloated more severely than uninoculated controls. The control cows sometimes bloated slightly, but only after a high concentrate-low roughage ration was fed for several weeks.

Bloat was produced in heifers inoculated with known species of bacteria

which demonstrated mucinolytic activity. Two species of lactobacilli which were found to be nonmucinolytic also produced bloat. These organisms were suspected of being mucinolytic when grown on selective media. Other nonmucinolytic organisms did not produce bloat.

Mucinolytic rumen organism H could not produce bloat in heifers fed a high roughage ration which enhances the secretion of saliva.

From the results of this study it was postulated that a lowered mucin content of the rumen resulting from reduced salivation during the feeding of low roughage-high concentrate rations might be a factor in the production of feedlot bloat in cattle. Destruction of mucin by mucinolytic bacteria, either present in the rumen or developed due to change of feeds, may be a second factor in the production of feedlot bloat.

High roughage rations appear to stimulate the secretion of such a large quantity of saliva that rumen inoculation of mucinolytic bacteria fail to degrade saliva rapidly enough to provoke bloat. Hence, these results support the hypothesis that a decrease in salivary secretion per se or a reduction in the saliva concentration of the rumen due to destruction of mucin permit the development of bloat on feeds containing frothing factors.

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THE ROLE OF MUCINOLYTIC BACTERIA IN FEEDLOT BLOAT

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Foaming of the rumen contents due to the presence of foaming agents in feeds and to a diminished salivary secretion has been recognized as a major factor in the etiology of legume and feedlot bloat. Since the effectiveness of saliva as an antifoaming agent in bloat was found to last only a few hours, it was conjectured that the natural antifoaming properties of saliva, might be destroyed in the rumen by mucinolytic bacteria, thus permitting bloat to ensue. An attempt was made at this station to determine whether mucinolytic organisms existed in the rumen. Several organisms which degraded salivary mucin were isolated from the rumen. When these bacteria were introduced into the rumens of cows grazing on a mature nonbloat provoking alfalfa pasture, bloat resulted in the majority of instances. In the light of these results, the studies reported herein were initiated to determine if mucinolytic organisms could provoke bloat in cattle fed a feedlot ration.

Three pairs of fistulated identical-twin cows, three pairs of nonfistulated identical-twin heifers, four dry dairy cows, and three beef heifers were used in this study.

One mucinolytic organism (designated as organism H) was used in the first seven experiments. Several other organisms: Staphylococcus albus, Staphylococcus aureus, Streptococcus faecalis, Sarcina lutea, Escherichia coli, Proteus vulgaris, Aerobacter aerogenes, Salmonella pullorum, Lactobacillus buchneri, Lactobacillus casei, Lactobacillus acidophilus, and Lactobacillus plantarum, both of rumen and nonrumen origin, were tested for mucinolytic activity using a carbon free medium containing saliva as the sole source of organic nutrients. Staphylococcus albus, Sarcina lutea, and Escherichia coli were found to be mucinolytic and the others nonmucinolytic. These organisms were used in later experiments.

The animals were maintained on a bloat provoking ration until bloat was

induced. The minimum amount of long alfalfa hay needed to prevent bloat was fed. After bloat ceased, one member of each identical-twin pair (or a member of each group when twin pairs were not used) was inoculated with one species of organism grown in nutrient broth for 36 to 48 hours at 37° C. The other member of the twin pair or the group served as a control. Experiments were replicated.

In seven experiments cows fed the feedlot bloat ration with hay and inoculated with mucinolytic rumen organism H bloated more severely than uninoculated controls. The control cows sometimes bloated slightly, but only after a high concentrate-low roughage ration was fed for several weeks.

Bloat was produced in heifers inoculated with known species of bacteria which demonstrated mucinolytic activity. Two species of lactobacilli (L. buchneri and L. plantarum) which were found to be nonmucinolytic also produced bloat. These organisms were suspected of being mucinolytic when grown on selective medium. Other nonmucinolytic organisms did not produce bloat.

Repeated inoculations of mucinolytic rumen organism H could not produce bloat in heifers fed a high roughage ration which enhances the secretion of saliva.

From the results of this study it was postulated that a lowered mucin content of the rumen resulting from reduced salivation during the feeding of low roughage-high concentrate rations might be a factor in the production of feedlot bloat in cattle. Destruction of mucin by mucinolytic bacteria, either present in the rumen or developed due to change of feeds, may be a second factor in the production of feedlot bloat in cattle.